Priority

The Examiner indicates that this application claims priority to copending Application No. 08/944,850 filed October 6, 1997 and PCT/US98/21193, flied October 6, 1998; however, the current status of the nonprovisional parent applications referenced should be included. Applicants submit that the term "copending" indicates that the parent applications are pending. As such, no amendment has been made to further clarify this issue. If the Examiner is wanting additional information, Applicants request clarification so that any necessary information can be obtained.

Drawings

Applicants appreciate that the drawings are considered informal and are objected to by the Draftsperson. Applicants will furnish formal drawings when the application is allowed.

Specification

The Examiner notes that "the characters 'A' and '@' are mediated by specific technical or otherwise, terms used by the Applicants." Applicants note that "A" and "@" were inadvertently inserted into the text flanking certain terms that were to be in quotations as a result of a word processing error that replaced the intended quotations with "A" and '@". Applicants have amended the specification herein to correct this inadvertent error. Applicants submit that no new matter is introduced by way of this amendment.

Response to Rejection under 35 USC § 112

Claims 16-44 are rejected under 35 USC § 112, second paragraph. Claim 16 is rejected as being incomplete. Basically the Examiner's position is that the claim lacks a positive contacting step between the array and the analyte. In response, Applicants note that the claims have

been amended to include the step of contacting the array with a composition comprising a target analyte. As such, Applicants respectfully request the Examiner to withdraw this rejection.

The Examiner indicates that claim 16 is confusing because it is unclear which "measurements" are being statistically analyzed. In response Applicants note that the claims have been amended for clarity and indicate the measurements to be statistically analyzed. As such, Applicants submit that the claims are not confusing and clearly specify which measurements are to be statistically analyzed.

The Examiner also directs Applicants to "[s]ee also claims 25, 27, 34, 36, 37." Applicants interpret this reference to other claims to mean that the Examiner is repeating a rejection of these claims similar to the rejection of claim 16. In response, Applicants note that the claims (or the base claim to which a dependent claim refers) have been amended to clarify the allegedly confusing language.

Claim 16 (see also claims 25 and 27) are vague and indefinite because they do not recite a positive contacting step. As noted above, the claims have been amended to include a step of contacting the array with a composition comprising a target analyte.

The Examiner notes that claims 16, 25 and 27 are unclear in the recitation of "target analyte" because the specification makes reference to a "reference analyte". the Examiner points to p. 40, line 1 (sic; line 6). However, Applicants draw the Examiner's attention to p. 42, line 7 where the term "target analyte" is described. Applicants note that the term "target analyte" is flanked by the characters "A" and "@". As noted above, these typographical characters are the result of an unintentional word processing error that replaced quotation marks with these characters. These errors are corrected by amendment herein. As such, Applicants submit that the specification clearly describes "target analyte". Accordingly, Applicants respectfully request the Examiner to withdraw this rejection.

Claims 17 and 18 are rejected as having improper antecedent basis. Applicant note that the rejection is moot in light of the amendment of the claims.

Claims 17 and 26 are rejected as vague and indefinite because the term "adjusted" is allegedly unclear. Applicants respectfully traverse.

As noted throughout the specification, the baseline of an optical response of a sensor element may be adjusted. Applicants submit that the specification sets forth the meaning of baseline adjusted. Moreover, Applicants submit that one of ordinary skill in the art recognizes the meaning of the term "baseline adjusted". The skilled artisan would appreciate that this can be accomplished by a variety of ways including (but certainly not limited to) adjusting a detector so as to eliminate any detectable baseline measurements or by measuring the baseline and subtracting this measurement from experimentally obtained measurements. Moreover, Applicants submit that such baseline adjustment is set forth in the specification.

For example, as noted at p. 46, line 21-22, "[b]aseline control measurements were performed with high purity, Ultra Zero grade air". Clearly, this demonstrates a determination of the baseline or background of the system. P. 46, lines 30-31 note that the "air pulse data is then subtracted from the vapor pulse data to subtract the background due to the air alone." As such, Applicants submit that this represents one example of baseline adjustment. In addition, Applicants note that p. 49, lines 5-6 of the specification sets forth yet another example of baseline adjustment where "the standardized optical responses are adjusted to start at a value of 0.0".

Accordingly, Applicants submit that the specification and claims clearly set forth the meaning of "adjustment" of the baseline optical response. Applicants respectfully request the Examiner to withdraw this rejection.

Claims 18 and 19 are rejected as indefinite and confusing in reciting a factor of at least 10 or

100, respectively, because it is unclear as to how the factor of at least 10 or 100 is increased or reduced, respectively. Specifically, the Examiner notes that it is unclear if the factor is increased or reduced "i.e. cumulatively, multiply (exponentially)." Applicants respectfully traverse the rejection.

Applicants submit that the skilled artisan would understand the term "factor of 10" or "factor of 100" to be synonymous with 10-fold or 100-fold, respectively. Thus, by increasing the signal-to-noise ratio by a factor of 10, one would increase the signal-to-noise ratio 10-fold. In fact, at p. 56, lines 20-21, the specification recites that the signal-to-noise ratio is increased "ten-fold or greater". Likewise, by reducing the detection limit by a factor of 100, one would reduce the detection limit by 100-fold. Again, the specification clearly notes at p. 56, lines 30-31, that improvements "up to a hundred fold, in the detection limit" an be obtained by the claimed methods. As such, Applicants submit that the claims clearly set forth the methods. Applicants respectfully request the Examiner to withdraw the rejection.

Claim 20 is rejected as being vague and indefinite because the relationship between the "beads" and the "sensor elements" in claim 16 is unclear. Applicants submit that the claim as amended clearly sets forth the relationship between the "beads" and the "sensor elements". As such, Applicants respectfully request the Examiner to withdraw this rejection.

Claim 16 is rejected under 35 U.S.C. 112, first paragraph because the Examiner failed to find literal support for a "method for increasing the signal-to-noise ratio in the characteristic optical response". Applicants respectfully traverse.

As noted at p. 40, lines 19-20, summation of the optical response "can result in an increase in the signal-to-noise ratio". As such, Applicants submit that the specification provides support for a method for increasing the signal-to-noise ration. Applicants respectfully request the Examiner to withdraw the rejection.

Response to Rejections under 35 USC § 103

Claims 16-17, 20-39 and 40-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Walt et al. (US 6,023,540 (Walt)).

While the Examiner has indicated that the claims are rejected under 35 USC 103 (a), Applicants note that as a result of the present filing of a continuing application under 37 C.F.R. 1.53(d), the present application is subject to the new rules for applications filed subsequent to November 29, 1999. As such, Applicants submit that Walt is not prior art against the present application. That is, revised 35 U.S.C. 103(c) provides that "[s]ubject matter developed by another person, which qualifies as prior art only under one or more subsections (e), (f), and (g) of section 102...shall not preclude patentability under this section where the subject matter and the claimed invention were...subject to an obligation of assignment to the same person."

The Walt patent issued on February 8, 2000 and has a filing date of March 14, 1997. The present application was filed April 6, 1999, and claims priority to US application 080/944,850, filed October 6, 1997. As such, the present application was filed prior to the issuance of the Walt patent, but subsequent to the filing of the Walt patent, a potential 102(e) situation. However, Applicants submit that the Walt patent and the present application are assigned to the same entity, namely Trustees of Tufts College. In support of this; Applicants are submitting copies of the assignment of the present application and a copy of the front page of the Walt patent which indicates that Trustees of Tufts College is the assignee. In addition, Applicants draw the Examiner's attention to reel/frame 8619/0496 which documents the assignment of the Walt patent. In addition, Applicants respectfully direct the Examiner to reel/frame 010384/0082 which documents the assignment of the present case. Since both the present application and the Walt patent were subject to an obligation of assignment at the time the present invention was made, Applicants submit that the Walt patent is not a proper reference upon which to base a rejection under 103(a). Accordingly, Applicants respectfully request the Examiner to withdraw this rejection.

Claims 16, 23-25, 27-30 and 32-43 are rejected under 35 U.S.C. 103 as being unpatentable over Pinkel et al. (US 5,837,196 (Pinkel)). Basically the Examiner indicates that Pinkel teaches methods of using a fiber optic sensor for performing quantitative analysis of bioactive agents. The Examiner further indicates that although Pinkel

> is silent in specifically teaching the step of summing the optical responses as well as the statistical analysis and calculations of data obtained from individual or combinatorial optical signal measurements from sensor elements...it would have been obvious to obtain a summation of optical responses of at least two sensor elements...in order to establish a baseline optical response signature representative of the subpopulations. Further, statistical analysis strategies...are standard laboratory practice and a requirement in optimization procedures.

As such, the Examiner asserts that it would have been obvious to use statistical analysis strategies known and conventionally used in prior art. Applicants respectfully traverse the rejection.

Pinkel teaches sensors prepared by bundling individually derivatized optical fibers and methods of detecting target molecules with such individually derivatized optical fiber strands. However, Pinkel fails to teach or suggest a method for increasing the signal-to-noise ratio of a sensor array as presently claimed. In addition, Pinkel fails to teach a method of amplifying the optical response of a sensor array as presently claimed. Moreover, as noted by the Examiner, Pinkel is silent with respect to any teaching of summing the optical responses of a sensor array and performing statistical analysis.

As the Examiner is aware, there are three requirements to establish a *prima facie* case of obviousness. These include that "there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations" (MPEP § 2143).

First, Applicants submit that Pinkel fails to provide any motivation for the modification of Pinkel to reach the presently claimed invention. That is, a valid rejection under 35 U.S.C. § 103 based upon a single prior art reference must be supported by some suggestion of the claimed invention or motivation to reach the claimed invention which is found in that single prior art reference. In re Laskowski, 10 USPQ2d 1397 (CAFC 1989). Applicants submit that there is no teaching in Pinkel that would motivate one of ordinary skill in the art to modify the teaching of Pinkel to reach the claimed invention, and in fact the Examiner agrees. That is, as noted above, Pinkel is silent with respect to teaching summing. Rather, the Examiner appears to be using obviousness to provide the motivation to modify the references, which is legally incorrect. The mere fact that a reference can be modified does not render the resultant modification obvious unless the prior art also provides the motivation to modify the reference to arrive at the claimed invention. *In re Mills*, 16 USPQ2d 1430 (Fed. Cir. 1990); MPEP § 2143.01. In the present case, even as admitted by the Examiner, the prior art (Pinkel) fails to disclose summing responses in order to increase the signal-to-noise ratio of a sensor. As such, the Pinkel fails to provide the requisite motivation for the combination of the reference to reach the claimed invention.

Second, Applicants submit that there is no teaching in Pinkel that would provide one of ordinary skill in the art a reasonable expectation of success in practicing the claimed invention. That is, Pinkel fails to provide any teaching that would indicate that summing of the responses of sensor elements would result in an increase in the signal-to-noise ratio of the response or result in the amplification of the signal. Moreover, Applicants submit that the Office Action fails to provide any evidence that would indicate that the skilled artisan would have a reasonable expectation of success in practicing the claimed invention upon reading the Pinkel reference.

Finally, Applicants submit that Pinkel fails to set forth teach element of the claims. That is, as described above, Pinkel is silent with respect to summing the optical responses of sensor elements and/or performing statistical analysis on measurements obtained from the responses of the sensor elements. Applicants note that summing and/or statistical analysis are elements

of the pending claims. As such, Pinkel fails to teach each element of the claims.

Accordingly, as set forth above, Pinkel fails to provide the motivation to modify the reference to reach the claimed invention. In addition, Pinkel fails to provide a reasonable expectation of success in practicing the claimed invention. Finally, Applicants submit that Pinkel fails to teach each element of the claims. As such, Applicants submit that a *prima facie* case of obviousness has not been established and Applicants respectfully request the Examiner to withdraw the rejection.

Claims 18 and 19 are rejected under 35 U.S.C. 103 (a) as being unpatentable over Walt et al (6,023,540). As noted above, the present application has been re-filed under 37 CFR 1.53(d). As such, in accordance with 35 USC 103(c), Walt is not prior art against the present application.

Claims 18 and 19 are rejected under 35 U.S.C. 103 (a) as being unpatentable over Pinkel et al. (5,837,196).

The Examiner indicates that although Pinkel fails "to disclose increasing signal-to-noise ratio by a factor of at least 10 and reducing analyte detection limit by a factor of at least 100", it is maintained that such increasing and reducing are "all result effective variables which the prior art references have shown may be obtained via optimization procedures in order to achieve optimum results." The Examiner cites *Application of Boesch*, 617 F.2d 272, 276, 205 USPQ 215, 218-219 (C.C.P.A. 1980) in support of the argument that the "discovery of an optimum value of a result effective variable in a known process is ordinarily within the skill of the art." Applicants respectfully traverse the rejection.

Initially, Applicants distinguish Boesch on the following ground. In Boesch, a claim was held obvious over two prior art references that "disclose alloys having compositional limits overlapping those of the claimed alloys." *Application of Boesch*, 617 F.2d 272, 276, 205 USPQ

215, 218 (C.C.P.A. 1980). That is, each of the prior art references taught compositions similar to those claimed by Applicant, although Applicant modified or optimized the amount of the compositions. Of note, the Applicants' claimed compositions overlapped with limits described in the prior art.

In contrast, Pinkel fails to disclose the presently claimed methods. As such, Applicants submit that the presently claimed invention is not the result of optimizing a result effective variable in a known process. That is, prior to the Applicant's invention, the method was not a known process. As such, Applicants submit that the claims are not obvious over Pinkel. Applicants respectfully request the Examiner to withdraw the rejection.

Claims 16, 25, 27 and 32-39 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rushbrook et al (GB 2294319A (Rushbrook)). Basically, the Examiner suggests that Rushbrook teaches detecting the position of labeled material by repetitively imaging the sample and performing measurements on the data signals and comparing the measurements with threshold to distinguish clusters resulting from optical signals from the remainder of the sample. The Examiner notes that Rushbrook is silent in teaching statistical analysis and calculations of data. The Examiner indicates that it would have been obvious for one of ordinary skill in the art to modify the teachings of Rushbrook to reach the claimed invention because statistical analysis and calculations are standard laboratory procedures required in optimization procedures. Applicants respectfully traverse.

Rushbrook provides for detecting the presence and position of labeled material by repeatedly scanning and imaging a sample and comparing the image with the image of the threshold image. However, Applicants note, as the Examiner points out, that Rushbrook is "silent in specifically teaching statistical analysis and calculations" (p. 12 of the Office Action). Moreover, Applicants note that Rushbrook fails to teach or suggest a method of increasing the signal-to-noise ratio in the optical response of a sensor by obtaining measurements from at least two sensor elements of a subpopulation and summing the measurements.

As noted above, there are three requirements to establish a *prima facie* case of obviousness. Applicants submit that Rushbrook fails to provide any motivation to modify the teachings in Rushbrook to reach the claimed invention. Rather, the Examiner appears to be using obviousness to provide the motivation to modify the references, which is legally incorrect. As noted above, the mere fact that a reference can be modified does not render the resultant modification obvious unless the prior art also provides the motivation to modify the reference to arrive at the claimed invention. see *In re Mills*, supra. In the present case, even as admitted by the Examiner, Rushbrook fails to disclose a method as presently claimed. That is, nowhere in Rushbrook is there a teaching or suggestion that would motivate one of ordinary skill in the art to modify the method as disclosed in Rushbrook, which is directed to detecting the presence and position of a labeled material, to reach a method as claimed, namely a method of increasing the signal-to-noise ratio of a sensor, or amplifying the optical response of a sensor. As such, Rushbrook fails to provide the requisite motivation for the modification of the reference to reach the claimed invention.

In addition, Applicants submit that there is no teaching in Rushbrook that would have provided one of ordinary skill in the art with a reasonable expectation of success in practicing the method as claimed. That is, there is no teaching that would have suggested a reasonable expectation of increasing the signal-to-noise ratio of an optical sensor by summing the measurements and/or performing statistical analysis on the measurements.

Finally, Applicants submit that Rushbrook fails to teach each element of the claims. That is, as previously noted and pointed out by the Examiner, Rushbrook is silent in specifically teaching statistical analysis. In addition, Applicants submit that Rushbrook fails to teach summing the responses to increase the signal-to-noise ratio, or a sensor array comprising at least first and second subpopulations of sensor elements. While Applicants note that Rushbrook mentions labeling of target molecules with different labels and contacting the target with "DNA material located in discrete regions of the membrane", there is no teaching or suggestion that the "DNA material" includes first and second subpopulations of sensor elements. Accordingly, Applicants

submit that Rushbrook fails to teach each element of the claims.

In conclusion, Applicants submit that the Office Action has failed to establish a prima facie case of obviousness in light of Rushbrook. As noted above, Rushbrook fails to provide the motivation for modifying the teachings of Rushbrook to reach the claimed invention. In addition, Applicants submit that there is no teaching in Rushbrook that would provide one of ordinary skill in the art with a reasonable expectation of practicing the invention as claimed. Finally, Applicants note that Rushbrook fails to teach each element of the claims. As such, Applicants submit that the rejection is in error. Applicants respectfully request the Examiner to withdraw the rejection.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

CONCLUSION

The applicants submit that the claims are now in condition for allowance and an early notification of such is respectfully solicited. The Examiner is invited to contact the undersigned at 415-781-1989 if any issues may be resolved in that manner.

Respectfully submitted,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP

Dated: May 18, 2001

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at page 9, line19, has been amended as follows:

Fig. 21 depicts sequences used in the array (Table 1). Each probe has a [5=] 5'-(NH_2-$(CH_2)_6$-) functionality for cyanuric chloride activation and attachment to the microspheres. Each complementary target has a [5=] 5'-fluorescein label;

Paragraph beginning at page 11, line 31, has been amended as follows:

An additional benefit of the present invention is that it allows the synthesis of the bioactive agents (i.e. compounds such as nucleic acids and antibodies) to be separated from their placement on an array, i.e. the bioactive agents may be synthesized on the beads, and then the beads are randomly distributed on a patterned surface. Since the beads are self-encoded by having dyes present that have known responses to a reference analyte, this means that the array can later be [Adecoded@] "decoded", i.e. after the array is made, a correlation of the location of an individual site on the array with the bead or bioactive agent at that particular site can be made. This means that the beads may be randomly distributed on the array, a fast and inexpensive process as compared to either the *in situ* synthesis or spotting techniques of the prior art. Once the array is loaded with the beads, the array can be decoded, or can be used, with full or partial decoding occurring after testing, as is more fully outlined below.

Paragraph beginning at page 14, line 13, has been amended as follows:

--Accordingly, the present invention provides array compositions comprising at least a first substrate with a surface comprising individual sites. By [Aarray@] "array" herein is meant a plurality of bioactive agents in an array format; the size of the array will depend on the

composition and end use of the array. Arrays containing from about 2 different bioactive agents (i.e. different beads) to many millions can be made, with very large fiber optic arrays being possible. Generally, the array will comprise from two to as many as a billion or more, depending on the size of the beads and the substrate, as well as the end use of the array, thus very high density, high density, moderate density, low density and very low density arrays may be made. Preferred ranges for very high density arrays are from about 10,000,000 to about 2,000,000,000, with from about 100,000,000 to about 1,000,000,000 being preferred. High density arrays range about 100,000 to about 10,000,000, with from about 1,000,000 to about 5,000,000 being particularly preferred. Moderate density arrays range from about 10,000 to about 50,000 being particularly preferred, and from about 20,000 to about 30,000 being especially preferred. Low density arrays are generally less than 10,000, with from about 1,000 to about 5,000 being preferred. Very low density arrays are generally less than 1,000, with from about 10 to about 1000 being preferred, and from about 100 to about 500 being particularly preferred. In some embodiments, the compositions of the invention may not be in an array format; that is, for some embodiments, compositions comprising a single bioactive agent may be made as well. In addition, in some arrays, multiple substrates may be used, either of different or identical compositions. Thus for example, large arrays may comprise a plurality of smaller substrates.--

Paragraph beginning at page 15, line 4, was amended as follows:

The compositions comprise a substrate. By [Asubstrate@] "substrate" or [Asolid support@] "solid support" or other grammatical equivalents herein is meant any material that can be modified to contain discrete individual sites appropriate for the attachment or association of beads and is amenable to at least one detection method. As will be appreciated by those in the art, the number of possible substrates are very large, and include, but are not limited to, glass and modified or functionalized glass, plastics (including acrylics, polystyrene and copolymers of styrene and other materials, polypropylene, polyethylene, polybutylene, polyurethanes, [TeflonJ,] Teflon™ etc.), polysaccarides, nylon or nitrocellulose, resins, silica or

silica-based materials including silicon and modified silicon, carbon, metals, inorganic glasses, plastics, optical fiber bundles, and a variety of other polymers. In general, the substrates allow optical detection and do not appreciably fluorescese.

Paragraph beginning at page 16, line 24, was amended as follows:

In a preferred embodiment, the surface of the substrate is modified to contain chemically modified sites, that can be used to attach, either covalently or non-covalently, the microspheres of the invention to the discrete sites or locations on the substrate. [Achemically modified sites@] "Chemically modified sites" in this context includes, but is not limited to, the addition of a pattern of chemical functional groups including amino groups, carboxy groups, oxo groups and thiol groups, that can be used to covalently attach microspheres, which generally also contain corresponding reactive functional groups; the addition of a pattern of adhesive that can be used to bind the microspheres (either by prior chemical functionalization for the addition of the adhesive or direct addition of the adhesive); the addition of a pattern of charged groups (similar to the chemical functionalities) for the electrostatic attachment of the microspheres, i.e. when the microspheres comprise charged groups opposite to the sites; the addition of a pattern of chemical functional groups that renders the sites differentially hydrophobic or hydrophilic, such that the addition of similarly hydrophobic or hydrophilic microspheres under suitable experimental conditions will result in association of the microspheres to the sites on the basis of hydroaffinity. For example, the use of hydrophobic sites with hydrophobic beads, in an aqueous system, drives the association of the beads preferentially onto the sites. As outlined above, [Apattern@] "pattern" in this sense includes the use of a uniform treatment of the surface to allow attachment of the beads at discrete sites, as well as treatment of the surface resulting in discrete sites. As will be appreciated by those in the art, this may be accomplished in a variety of ways.

Paragraph beginning at page 17, line 8, was amended as follows:

The compositions of the invention further comprise a population of microspheres. [By Apopulation@] By "population" herein is meant a plurality of beads as outlined above for arrays. Within the population are separate subpopulations, which can be a single microsphere or multiple identical microspheres. That is, in some embodiments, as is more fully outlined below, the array may contain only a single bead for each bioactive agent; preferred embodiments utilize a plurality of beads of each type.

Paragraph beginning at page 17, line 14, was amended as follows:

By [Amicrospheres@ or Abeads@ or Aparticles@] "microspheres" or "beads" or "particles" or grammatical equivalents herein is meant small discrete particles. The composition of the beads will vary, depending on the class of bioactive agent and the method of synthesis. Suitable bead compositions include those used in peptide, nucleic acid and organic moiety synthesis, including, but not limited to, plastics, ceramics, glass, polystyrene, methylstyrene, acrylic polymers, paramagnetic materials, thoria sol, carbon [graphited] graphite, titanium dioxide, latex or cross-linked dextrans such as Sepharose, cellulose, nylon, cross-linked micelles and [teflon] Teflon™ may all be used.

Paragraph beginning at page 22, line 18, was amended as follows:

In a preferred embodiment, the microspheres further comprise a bioactive agent. By [Acandidate bioactive agent@ or Abioactive agent@ or Achemicla functionality@ or Abinding ligand@] "candidate bioactive agent" or "bioactive agent" or "chemical functionality" or "binding ligand" herein is meant as used herein describes any molecule, *e.g.*, protein, oligopeptide, small organic molecule, polysaccaride, polynucleotide, etc. which can be attached to the microspheres of the invention. It should be understood that the compositions of the invention have two primary uses. In a preferred embodiment, as is more fully outlined below, the compositions are used to detect the presence of a particular target analyte; for example, the presence or absence of a particular nucleotide sequence or a particular protein, such as an

enzyme, an antibody or an antigen. In an alternate preferred embodiment, the compositions are used to screen bioactive agents, i.e. drug candidates, for binding to a particular target analyte.

Paragraph beginning at page 23, line 13, was amended as follows:

In a preferred embodiment, the bioactive agents are proteins. By [Aprotein@] "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures. Thus "amino acid", or "peptide residue", as used herein means both naturally occurring and synthetic amino acids. For example, homo-phenylalanine, citrulline and norleucine are considered amino acids for the purposes of the invention. The side chains may be in either the (R) or the (S) configuration. In the preferred embodiment, the amino acids are in the (S) or L-configuration. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard *in vivo* degradations.

Paragraph beginning at page 23, line 30, was amended as follows:

In a preferred embodiment, the bioactive agents are peptides of from about 5 to about 30 amino acids, with from about 5 to about 20 amino acids being preferred, and from about 7 to about 15 being particularly preferred. The peptides may be digests of naturally occurring proteins as is outlined above, random peptides, or [Abiased@] "biased" random peptides. By [Arandomized@] "randomized" or grammatical equivalents herein is meant that each nucleic acid and peptide consists of essentially random nucleotides and amino acids, respectively. Since generally these random peptides (or nucleic acids, discussed below) are chemically synthesized, they may incorporate any nucleotide or amino acid at any position. The synthetic process can be designed to generate randomized proteins or nucleic acids, to allow the formation of all or most of the possible combinations over the length of the sequence, thus

forming a library of randomized bioactive proteinaceous agents.

Paragraph beginning at page 24, line 27, was amended as follows:

In a preferred embodiment, the bioactive agents are nucleic acids (generally called [Aprobe nucleic acids@ or Acandidate probes@] "probe nucleic acids" or "candidate probes" herein). By [Anucleic acid@] "nucleic acid" or "oligonucleotide" or grammatical equivalents herein means at least two nucleotides covalently linked together. A nucleic acid of the present invention will generally contain phosphodiester bonds, although in some cases, as outlined below, nucleic acid analogs are included that may have alternate backbones, comprising, for example, phosphoramide (Beaucage, *et al.*, Tetrahedron **49**(10):1925 (1993) and references therein; Letsinger, J. Org. Chem., **35**:3800 (1970); Sprinzl, *et al.*, Eur. J. Biochem., **81**:579 (1977); Letsinger, *et al.*, Nucl. Acids Res., **14**:3487 (1986); Sawai, *et al.*, Chem. Lett., 805 (1984); Letsinger, *et al.*, J. Am. Chem. Soc., **110**:4470 (1988); and Pauwels, *et al.*, Chemica Scripta, **26**:141 (1986)), phosphorothioate (Mag, *et al.*, Nucleic Acids Res., **19**:1437 (1991); and U.S. Patent No. 5,644,048), phosphorodithioate (Briu, *et al.*, J. Am. Chem. soc., **111**:2321 (1989)) O-methylphophoroamidite linkages (see Eckstein, Oligonucleotides and Analogues: A Practical Approach, Oxford University Press), and peptide nucleic acid backbones and linkages (see Egholm, J. Am. Chem. Soc., **114**:1895 (1992); Meier, *et al.*, Chem. Int. Ed. Engl., **31**:1008 (1992); Nielsen, Nature, **365**:566 (1993); Carlsson, *et al.*, Nature, **380**:207 (1996), all of which are incorporated by reference)). Other analog nucleic acids include those with positive backbones (Denpcy, *et al.*, Proc. Natl. Acad. Sci. USA, **92**:6097 (1995)); non-ionic backbones (U.S. Patent Nos. 5,386,023; 5,637,684; 5,602,240; 5,216,141; and 4,469,863; Kiedrowshi, *et al.*, Angew. Chem. Intl. Ed. English, **30**:423 (1991); Letsinger, *et al.*, J. Am. Chem. Soc., **110**:4470 (1988); Letsinger, *et al.*, Nucleosides & Nucleotides, **13**:1597 (1994); Chapters 2 and 3, ASC Symposium Series 580, [Acarbohydrate Modifications in Antisense Research@] Carbohydrate Modifications in Antisense Research, Ed. Y.S. Sanghui and P. Dan Cook; Mesmaeker, *et al.*, Bioorganic & Medicinal Chem. Lett., **4**:395 (1994); Jeffs, *et al.*, J. Biomolecular NMR, **34**:17 (1994); Tetrahedron Lett., **37**:743 (1996)) and non-ribose backbones,

including those described in U.S. Patent Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7, ASC Symposium Series 580, [Acarbohydrate Modifications in Antisense Research@] Carbohydrate Modifications in Antisense Research, Ed. Y.S. Sanghu and P. Dan Cook. Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids (see Jenkins, *et al.*, Chem. Soc. Rev., (1995) pp. 169-176). Several nucleic acid analogs are described in Rawls, C & E News, June 2, 1997, page 35. All of these references are hereby expressly incorporated by reference. These modifications of the ribose-phosphate backbone may be done to facilitate the addition of additional moieties such as labels, or to increase the stability and half-life of such molecules in physiological environments. In addition, mixtures of naturally occurring nucleic acids and analogs can be made. Alternatively, mixtures of different nucleic acid analogs, and mixtures of naturally occurring nucleic acids and analogs may be made. The nucleic acids may be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA or a hybrid, where the nucleic acid contains any combination of deoxyribo- and ribo-nucleotides, and any combination of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xanthanine, hypoxanthanine, isocytosine, isoguanine, and basepair analogs such as nitropyrrole and nitroindole, etc.

Paragraph beginning at page 25, line 31, was amended as follows:

As described above generally for proteins, nucleic acid bioactive agents may be naturally occurring nucleic acids, random nucleic acids, or [Abiased@] "biased" random nucleic acids. For example, digests of procaryotic or eukaryotic genomes may be used as is outlined above for proteins.

Paragraph beginning at page 26, line 1, was amended as follows:

In general, probes of the present invention are designed to be complementary to a target sequence (either the target analyte sequence of the sample or to other probe sequences,

as is described herein), such that hybridization of the target and the probes of the present invention occurs. This complementarity need not be perfect; there may be any number of base pair mismatches that will interfere with hybridization between the target sequence and the single stranded nucleic acids of the present invention. However, if the number of mutations is so great that no hybridization can occur under even the least stringent of hybridization conditions, the sequence is not a complementary target sequence. Thus, by [Asubstantially complementary@] "substantially complementary" herein is meant that the probes are sufficiently complementary to the target sequences to hybridize under the selected reaction conditions. High stringency conditions are known in the art; see for example Maniatis et al., Molecular Cloning: A Laboratory Manual, 2d Edition, 1989, and Short Protocols in Molecular Biology, ed. Ausubel, et al., both of which are hereby incorporated by reference. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, [Aoverview of principles of hybridization and the strategy of nucleic acid assays@] "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30C for short probes (e.g. 10 to 50 nucleotides) and at least about 60C for long probes (e.g. greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. In another embodiment, less stringent hybridization conditions are used; for example, moderate or low stringency conditions may be used, as are known in the art; see Maniatis and Ausubel, supra, and Tijssen, supra.

Paragraph beginning at page 26, line 28, was amended as follows:

The term [>target sequence@] "target sequence" or grammatical equivalents herein means a nucleic acid sequence on a single strand of nucleic acid. The target sequence may be a portion of a gene, a regulatory sequence, genomic DNA, cDNA, RNA including mRNA and rRNA, or others. It may be any length, with the understanding that longer sequences are more specific. As will be appreciated by those in the art, the complementary target sequence may take many forms. For example, it may be contained within a larger nucleic acid sequence, i.e. all or part of a gene or mRNA, a restriction fragment of a plasmid or genomic DNA, among others. As is outlined more fully below, probes are made to hybridize to target sequences to determine the presence or absence of the target sequence in a sample. Generally speaking, this term will be understood by those skilled in the art.

Paragraph beginning at page 31, line 20, was amended as follows:

Fig. 4 illustrates how the microwells 250 are formed and microspheres 10 placed in the microwells. In one embodiment, a 1 mm hexagonally-packed imaging fiber bundle 202 was employed comprising approximately 20,600 individual optical fibers having cores approximately [3.7m] 3.7 μm across (Part No. ET26 from Galileo Fibers, Sturbridge, MA). Typically, the cores of each fiber are hexagonally shaped as a result of glass hardness and drawing during fiber fabrication. In some cases, the shape can be circular, however.

Paragraph beginning at page 31, line 27, was amended as follows:

In step 270, both the proximal 214 and distal 212 ends of the fiber bundle 202 are successively polished on [12 m, 9 m, 3 m, 1 m, and 0.3 m] 12 μm, 9 μm, 3 μm, 1 μm, and 0.3 μm lapping films. Subsequently, the ends can be inspected for scratches on a conventional atomic force microscope. In step 272, etching is performed on the distal end 212 of the bundle 202. A solution of 0.2 grams NH_4F (ammonium fluoride) with 600 μl dH_2O and 100 μl of HF

(hydrofluoric acid), 50% stock solution, may be used. The distal end 212 is etched in this solution for a specified time, preferably approximately 80 seconds.

Paragraph beginning at page 32, line 6, was amended as follows:

As illustrated in Figs. 5A and 5B, the foregoing procedure produces microwells by the anisotropic etching of the fiber cores 254 favorably with respect to the cladding 256 for each fiber of the bundle 202. The microwells have approximately the diameter of the cores 254, [3.7 m] 3.7 μm. This diameter is selected to be slightly larger than the diameters of the microspheres used, [3.1m] 3.1 μm, in the example. The preferential etching occurs because the pure silica of the cores 254 etches faster in the presence of hydrofluoric acid than the germanium-doped silica claddings 256.

Paragraph beginning at page 33, line 22, was amended as follows:

In addition, since the size of the array will be set by the number of unique optical response signatures, it is possible to [Areuse@] "reuse" a set of unique optical response signatures to allow for a greater number of test sites. This may be done in several ways; for example, by using a positional coding scheme within an array; different sub-bundles may reuse the set of optical response signatures. Similarly, one embodiment utilizes bead size as a coding modality, thus allowing the reuse of the set of unique optical response signatures for each bead size. Alternatively, sequential partial loading of arrays with beads can also allow the reuse of optical response signatures.

Paragraph beginning at page 33, line 30, was amended as follows:

In a preferred embodiment a spatial or positional coding system is done. In this embodiment, there are sub-bundles or subarrays (i.e. portions of the total array) that are utilized. By analogy with the telephone system, each subarray is an [Aarea code@] "area

code", that can have the same tags (i.e. telephone numbers) of other subarrays, that are separated by virtue of the location of the subarray. Thus, for example, the same unique dye/bead combinations can be reused from bundle to bundle. Thus, the use of 50 unique tags in combination with 100 different subarrays can form an array of 5000 different bioactive agents. In this embodiment, it becomes important to be able to identify one bundle from another; in general, this is done either manually or through the use of marker beads, i.e. beads containing unique tags for each subarray.

Paragraph beginning at page 34, line 19, was amended as follows:

In a preferred embodiment, the coding and decoding is accomplished by sequential loading of the microspheres into an array. As outlined above for spatial coding, in this embodiment, the optical response signatures can be [Areused@] "reused". In this embodiment, the library of microspheres each comprising a different bioactive agent (or the subpopulations each comprise a different bioactive agent), is divided into a plurality of sublibraries; for example, depending on the size of the desired array and the number of unique tags, 10 sublibraries each comprising roughly 10% of the total library may be made, with each sublibrary comprising roughly the same unique tags. Then, the first sublibrary is added to the fiber optic bundle comprising the wells, and the location of each bioactive agent is determined, using its optical response signature. The second sublibrary is then added, and the location of each optical response signature is again determined. The signal in this case will comprise the first optical response signature and the [Asecond@] "second" optical response signature; by comparing the two matrices the location of each bead in each sublibrary can be determined. Similarly, adding the third, fourth, etc., sublibraries sequentially will allow the array to be filled.

Paragraph beginning at page 39, line 20, was amended as follows:

The arrays of the present invention are constructed such that information about the identity of the bioactive agent is built into the array, such that the random deposition of the

beads on the surface of the substrate can be [Adecoded@] "decoded" to allow identification of the bioactive agent at all positions. This may be done in a variety of ways.

Paragraph beginning at page 40, line 4, was amended as follows:

In a preferred embodiment, the beads are loaded onto the substrate and then the array is decoded, prior to running the assay. This is done by detecting the optical response signature associated with the bead at each site on the array upon exposure to a reference analyte. This may be done all at once, if unique optical signatures are used, or sequentially, as is generally outlined above for the [Areuse@] "reuse" of sets of optical signatures. Alternatively, full or partial decoding may occur after the assay is run.

Paragraph beginning at page 42, line 6, was amended as follows:

In a preferred embodiment, the compositions are used to probe a sample solution for the presence or absence of a target analyte. By [Atarget analyte@ or Aanalyte@] "target analyte" or "analyte" or grammatical equivalents herein is meant any atom, molecule, ion, molecular ion, compound or particle to be either detected or evaluated for binding partners. As will be appreciated by those in the art, a large number of analytes may be used in the present invention; basically, any target analyte can be used which binds a bioactive agent or for which a binding partner (i.e. drug candidate) is sought.

Paragraph beginning at page 43, line 25, was amended as follows:

The present invention also finds use as a methodology for the detection of mutations or mismatches in target nucleic acid sequences. For example, recent focus has been on the analysis of the relationship between genetic variation and phenotype by making use of polymorphic DNA markers. Previous work utilized short tandem repeats (STRs) as polymorphic positional markers; however, recent focus is on the use of single nucleotide polymorphisms

(SNPs), which occur at an average frequency of more than 1 per kilobase in human genomic DNA. Some SNPs, particularly those in and around coding sequences, are likely to be the direct cause of therapeutically relevant phenotypic variants. There are a number of well known polymorphisms that cause clinically important phenotypes; for example, the apoE2/3/4 variants are associated with different relative risk of [Alzheimer=s] Alzheimer's and other diseases (see Cordor et al., Science 261 (1993). Multiplex PCR amplification of SNP loci with subsequent hybridization to oligonucleotide arrays has been shown to be an accurate and reliable method of simultaneously genotyping at least hundreds of SNPs; see Wang et al., Science, 280:1077 (1998); see also Schafer et al., Nature Biotechnology 16:33-39 (1998). The compositions of the present invention may easily be substituted for the arrays of the prior art.

Paragraph beginning at page 44, line 12, was amended as follows:

In a preferred embodiment, the binding of the bioactive agent and the target analyte is specific; that is, the bioactive agent specifically binds to the target analyte. By [Aspecifically bind@] "specifically bind" herein is meant that the agent binds the analyte, with specificity sufficient to differentiate between the analyte and other components or contaminants of the test sample. However, as will be appreciated by those in the art, it will be possible to detect analytes using binding which is not highly specific; for example, the systems may use different binding ligands, for example an array of different ligands, and detection of any particular analyte is via its [Asignature@] "signature" of binding to a panel of binding ligands, similar to the manner in which [Aelectronic noses@] "electronic noses" work. This finds particular utility in the detection of chemical analytes. The binding should be sufficient to remain bound under the conditions of the assay, including wash steps to remove non-specific binding, although in some embodiments, wash steps are not desired; i.e. for detecting low affinity binding partners. In some embodiments, for example in the detection of certain biomolecules, the dissociation constants of the analyte to the binding ligand will be less than about 10^{-4} -10^{-6} M^{-1}, with less than about 10^{-5} to 10^{-9} M^{-1} being preferred and less than about 10^{-7} -10^{-9} M^{-1} being particularly preferred.

Paragraph beginning at page 47, line 21, was amended as follows:

Data analysis: In the data analysis portion, pre-selected segments taken from a previously collected [Afocus@] "focus" image are transferred to the sequence of images collected. These segments, drawn by the user, allow the mean pixel intensity to be measured in particular regions throughout the image field. Typically, they are drawn over individual pixels of a fiber optic sensor array, each of which contains a bead. The script then enters a loop that steps through each frame, measuring the mean pixel intensity within each segment, and placing the values in data columns. The resulting columns can then be plotted to yield the temporal response of each bead of interest. Before plotting, however, responses are [Astandardized@] "standardized" by dividing the data for each bead response by its first point. Thus, all responses can be normalized to start at a value of 1.0.

Paragraph beginning at page 47, line 31, was amended as follows:

Redundancy: As shown in the Examples, the present invention shows that building redundancy into an array gives several significant advantages, including the ability to make quantitative estimates of confidence about the data and significant increases in sensitivity. Thus, preferred embodiments utilize array redundancy. As will be appreciated by those in the art, there are at least two types of redundancy that can be built into an array: the use of multiple identical sensor elements [(termed herein Asensor redundancy@)] (termed herein "sensor redundancy"), and the use of multiple sensor elements directed to the same target analyte, but comprising different chemical functionalities [(termed herein Atarget redundancy@)] (termed herein "target redundancy"). For example, for the detection of nucleic acids, sensor redundancy utilizes of a plurality of sensor elements such as beads comprising identical binding ligands such as probes. Target redundancy utilizes sensor elements with different probes to the same target: one probe may span the first 25 bases of the target, a second probe may span the second 25 bases of the target, etc. By building in either or both of these types of redundancy into an array, significant benefits are obtained. For example, a

variety of statistical mathematical analyses may be done.

Paragraph beginning at page 52, line 5, was amended as follows:

In this embodiment, a plurality of different sensor elements may be used, with from about 2 to about 20 being preferred, and from about 2 to about 10 being especially preferred, and from 2 to about 5 being particularly preferred, including 2, 3, 4 or 5. [Howeve] However, as above, more may also be used, depending on the application.

Paragraph beginning at page 52, line 12, was amended as follows:

One benefit of the sensor element summing (referred to herein as [Abead summing@] "bead summing" when beads are used), is the increase in sensitivity that can occur. As shown in Example 19, detection limits in the zeptomole range can be observed.

Paragraph beginning at page 59, line 5, was amended as follows:

Before attaching oligonucleotides to the microspheres, a family of dye-encoded microspheres was created. Fluorescent dyes were used to encode the microspheres. Europium(III)thenoyltrifluoro-acetonate-$3H_2O$ ($_{ex}/_{em}$=365/615) (Eu-dye), ($_{ex}/_{em}$=620/700) and 5-(and -6)-carboxytetramethyl-rhodamine, succinimidyl ester ($_{ex}/_{em}$=535/580) (TAMRA, SE) were chosen for this demonstration. The dyes were incorporated by exploiting the chemical properties of the amino-modified polystyrene microspheres as follows. 200-µL-aliquots of stock (1 mL of stock beads contains $5.8x10^9$ beads in 0.01% merthiolate in water) [3.1 m-diameter] 3.1 µm-diameter amine-modified poly(methylstyrene)divinylbenzene microspheres (Bangs Laboratories, Inc. Carmel, IN) were filtered and washed with dry THF then placed in a microcentrifuge tube. [200 L] 200 µL of europium(III)thenoyltrifluoroacetonate-$3H_2O$ [Eu-dye (Acros)] dye in THF was added to the beads. Eu-dye concentrations of 0, 0.001, 0.01, 0.025,

0.05, 0.1, 0.5, and 1 M were used. The microsphere/dye suspension was shaken (VWR Vortex Genie II) for 2 h. The suspensions were filtered separately (Millipore Type HVLP) and washed thoroughly with MeOH. The beads were stored in 0.0% Tween (essential for preparation and storage to prevent the beads from clumping together) in ultrapure water until use.

Paragraph beginning at page 59, line 20, was amended as follows:

Alternatively, external encoding was done. [Ten L] 10 µL of stock beads were rinsed (all rinsing procedure entailed placing the centrifuge tube containing the beads and solution into a microcentrifuge at 8000 rpm for 3 min, and liquid over the beads was removed using a pipette) with BT buffer (0.1 M boric acid, 0.1 M NaOH, 0.13 M $NaHCO_3$, 0.01% Tween, pH 9). The beads were suspended in [100 L BT buffer then 5 L] 100 µL BT buffer then 5 µL of dye solution [Cy5 (Amersham) or TAMRA(Molecular Probes)] in DMF was added. Cy5 concentrations of 0, 0.01, 0.05, 0.1, 0.3 mM and TAMRA concentrations of 0, 0.1, 0.4, and 3 mM were used. The beads were shaken for 2 h then rinsed three times with BT buffer then three times with PBST buffer (0.01 M phosphate buffer saline, 0.0% Tween, pH 7.4).

Paragraph beginning at page 59, line 29, was amended as follows:

The polystyrene microspheres swell in tetrahydrofuran (THF) enabling a dye to penetrate the microsphere and become entrapped with the microsphere contracts. The absorption and emission spectra of the dyes are not compromised within the [microsphere=s] microsphere's environment and their concentration remains constant over time. Eight distinguishable microsphere families were prepared by entrapping varying Eu-dye concentrations inside the microspheres. In addition to internal entrapment, the [microspheres=amine-modified] microspheres' amine-modified surface permitted coupling to amine-reactive dyes. Different concentrations of Cy5 and TAMRA were then attached to the surface amine groups of the eight Eu-dye beads. A library of 100 spectroscopically-distinguishable microsphere types was prepared using various combinations of the three dyes.

Microsphere encoding was carried out prior to oligonucleotide attachment because reaction with the amine reactive dyes after probe attachment affected the hybridization reaction. On the other hand, the oligonucleotide probes on the surface of the microspheres are not affected by subsequent internal encoding with Eu-dye.

Paragraph beginning at page 60, line 13, was amended as follows:

DNA probes were synthesized with a 5'-amino-C6 modifier (Glen Research) in the Tufts Physiology Department using an ABI synthesizer. 20 nmol of the 5'-amino-terminal oligonucleotide probe were dissolved in [180 L] 180 µL of 0.1 M sodium borate buffer (SBB pH 8.3). Oligonucleotide activation was initiated by adding 40 nmol of cyanuric chloride in [40 L] 40 µL of acetonitrile. After 1 h, unreacted cyanuric chloride was removed by three cycles of centrifugal ultrafiltration (Microcon 3, Amicon) and recovered in [200 L] 200 µL of 0.1 M SBB.--

Paragraph beginning at page 60, line 20, was amended as follows:

DNA functionalization. [Five L] Five µL of stock beads were rinsed with 0.02 M phosphate buffer (pH 7). [150 L] 150 µL of 5% glutaraldehyde in phosphate buffer was added to the beads. The beads were shaken for 1 h then rinsed three times with phosphate buffer. [150 L] 150 µL of 5% polyethyleneimine (PEI) was then added to the beads. The beads were shaken for 1 h then rinsed three times with phosphate buffer then three times with 0.1 M SBB (sodium borate buffer, pH 8.3). [100 L of 150 M] 100 µL of 150 µM cyanuric chloride-activated oligonucleotide probe in SBB buffer was added to the beads and shaken overnight. The probe solution was removed and saved to reuse. The beads were then rinsed three times with SBB buffer. Remaining amine groups were capped with succinic anhydride to prevent non-specific binding. [100 L] 100 µL of 0.1 M succinic anhydride in 90% DMSO, 10% SBB was added to the beads. The beads were shaken for 1 h then rinsed three times with SBB buffer then three times with TE buffer (10 mM Tris-HCL, pH 8.3, 1 mM EDTA, 0.1 M NaCl, 0.1% SDS).

Paragraph beginning at page 61, line 7, was amended as follows:

Microsphere-based fiber-optic sensors. Recently, we reported an array consisting of randomly distributed independently addressable micron-bead-sensors using an imaging-optical-fiber substrate. This system employed imaging fibers consisting of six thousand individually clad fibers that were melted and drawn together to form a coherent, [500-m] 500-µm diameter bundle. The compositional difference between the core and cladding of each fiber enables the cores to be etched selectively providing for the simultaneous formation of six thousand 3.5 µm-diameter wells in the surface of the fiber tip within seconds. See Michael et al., Anal. Chem 70:1242 (1998); Bronk et al., Anal. Chem. 67:2750 (1995) and Pantano et al., Chem. Materials 8:2832 (1996), all of which are incorporated by reference.

Paragraph beginning at page 61, line 16, was amended as follows:

Microwell formation. [500 m-diameter] 500 µm-diameter imaging fiber bundles containing $6x10^4$ individual fibers were chemically etched according to a previously detailed procedure; see Pantano et al. Chem. Materials 8:2832 (1996).
Please replace the paragraph beginning at page 61, line 16, with the following rewritten paragraph:

Paragraph beginning at page 61, line 20, was amended as follows:

Array formation. [Five L] Five µL of probe-functionalized beads were stored in [40 L] 40 µL of TE buffer. After selecting the desired probe-functionalized microspheres, [1 L] 1 µL of each bead solution was placed in a microcentrifuge tube and vortexed. [0.05 L] 0.05 µL of this mixture was placed onto the distal face of the imaging fiber containing the microwells. After evaporation of the solvent (approximately 3 min), the distal tip of the fiber is wiped with an anti-static swab to remove excess beads. When a new sensor is desired, sonicating the fiber tip for 3 min will regenerate the substrate.

Paragraph beginning at page 61, line 30, was amended as follows:

Controlling array formation. One of the primary advantages of this system is the ability to alter the types of microspheres contained in an array. Each milliliter of stock solution contains approximately $6x10^9$ microspheres enabling functionalization of billions of beads at once. Even after a 20x dilution a [1 L] 1 µL volume of microsphere solution contains enough beads to produce hundreds of different arrays. The density of microspheres in solution can control the number of occupied wells. With dilute solutions, empty wells remain after the initial array production. Additional microspheres bearing different probes can be added to the unoccupied sites or to the original solution at any time to create a more diverse array. If a different selection of beads is desired, sonicating the fiber tip removes all of the beads from the wells, enabling a new sensor array to be made in the same substrate.

Paragraph beginning at page 62, line 22, was amended as follows:

The fiber was not removed from the imaging system during testing, rinsing, or regeneration steps. The proximal tip of the fiber was secured in the fiber chuck of the imaging system and all solutions were brought to the [fiber=s] fiber's distal tip which housed the microbead sensors. Images acquired immediately prior to each test while the fiber tip was in buffer were subtracted from the response images. Background signals from empty wells were then subtracted from signals generated during each test.

Paragraph beginning at page 62, line 28, was amended as follows:

Hybridization in real time. Each [microsphere=s] microsphere's fixed position made possible a hybridization study in real time. A DNA array containing identical beads was placed on the imaging system. The distal tip of the fiber bearing the microsphere sensors was placed in a labeled-target solution. Emission from hybridizing labeled-target was captured every minute for several minutes. In the small region of the imaging fiber selected for this study, 70

microspheres held the probe complementary to the target in solution. Each microsphere was monitored independently and simultaneously. Signals from 40 beads were averaged to provide kinetic data. At relatively high concentrations of target, hybridization could be detected immediately, as seen by the steep slope of the data. While the sensor remained on the imaging system it was regenerated by dipping the fiber tip into a room-temperature formamide solution. The same microspheres were assayed several times by placing the regenerated fiber into the target solution and repeating the experiment. Consecutive studies show that the same sensor can be used for multiple tests.

Paragraph beginning at page 63, line 6, was amended as follows:

A background fluorescence image was acquired at wavelengths specific to fluorescein (excitation 490 nm emission 530 nm) with the fiber's distal tip in buffer. The [fiber=s] fiber's distal tip was then placed in [4 L] 4 µL of fluorescein-labeled target solution and one image was acquired every minute for 10 min. Subsequently, the fiber was dipped in 90% formamide in TE buffer at room temperature (rt) to regenerate the sensor and a background image was taken with the fiber in buffer. The fiber was again placed in the target solution where images were acquired for another 10 min interval.--

Paragraph beginning at page 63, line 23, was amended as follows:

The [fiber=s] fiber's distal tip was placed in [4 L] 4 µL of labeled-target solution for 5 min, rinsed with TE buffer, and a fluorescence image was acquired for 5 s. The fiber tip was then dipped in 90% formamide in TE (rt) to remove any hybridized target and regenerate the sensor. This procedure was repeated 100 times using the IL2 target and 5 times (intermittently during the IL2 tests) using the IL6 target.

Paragraph beginning at page 64, line 1, was amended as follows:

Microsphere sensitivity. The [fiber=s] fiber's distal tip was placed in [4 L] 4 µL of target solution until the hybridization signal to noise ratio was three. The signal was monitored after rinsing the fiber tip with TE buffer and acquiring a fluorescence image for 5 s while the fiber tip was in buffer. For the hour-long assays, a 0.6 mL centrifuge tube was filled and capped. A hole was drilled in the cap to enable the fiber tip to be placed in the target solution while preventing evaporation.

Paragraph beginning at page 64, line 7, was amended as follows:

Sensitivity with an intensified CCD camera. The 21-mer cystic fibrosis oligonucleotide probe and complement with F508C mutation (5'-TAT CAT CTG TGG TGT TTC CTA-3') were used for this study. The 5'-amino-terminal oligonucleotide probe was activated with 100 times excess of cyanuric chloride. The microspheres were incubated with 400 M cyanuric chloride-activated oligonucleotide. The fluorescein-labeled target was dissolved in 6X saline sodium phosphate EDTA buffer (SSPE) containing 0.1% SDS. The [fiber=s] fiber's distal tip was placed in [10 L] 10 µL of target solution during hybridization with occasional stirring. The distal tip was then washed with 6X SSPE and a fluorescence image was acquired with a Pentamax ICCD camera (Princeton Instruments) for 1 s while the fiber tip was in [120 L] 120 µL of 6X SSPE.

Paragraph beginning at page 64, line 30, was amended as follows:

The [fiber=s] fiber's distal tip was placed in a target solution for 5 min, rinsed with TE buffer, and fluorescence images were acquired for 5 s while the fiber was in buffer. Overlay segments were drawn to select the beads bearing a hybridization signal using IPLab software. These overlay segments were copies and pasted onto each of the encoding images and the selected [beads=] beads' identity was determined. The sensor was regenerated as described above and this procedure was repeated for each of the target solutions.

Paragraph beginning at page 65, line 1, was amended as follows:

Hybridization specificity in a multiplex assay. To demonstrate this microsphere array system, we first selected seven probes used in previous work (sequences 1-7 of Table 1, see figure 22). The DNA sequences chosen for the array were designed to be completely specific at room temperature. The signals at two of the three encoding wavelengths is used to positionally register the microspheres. After registration at the encoding wavelengths, the array is ready for use. The fiber tip is dipped into a fluorescent-labeled target solution. After a specified time, the fiber tip is removed from the target solution, rinsed with buffer, and placed in buffer solution. Microspheres bearing a complementary probe display a fluorescent signal due to the hybridized labeled target. Completely specific hybridizations for seven different targets in an array were observed. Replicates of each bead type located randomly within the array yield redundant information which contributes to the [array=s] array's reliability. Table 2 (Figure 23 shows the accuracy of the system to correctly identify the target.

Paragraph beginning at page 66, line 1, was amended as follows:

Sensitivity of the microspheres. There are three aspects to sensitivity: sample volume, target concentration, and absolute number of target molecules. The smaller the volume required, the less a sample needs to be amplified for detection since the same number of absolute target molecules in a smaller volume generates a higher local concentration. Sample volumes as small as [4 L] 4 µL are required with this system since only the tip of the [500 m-diameter] 500 µm-diameter fiber is dipped into the solution. Typically, we use [10 L] 10 µL volumes for easier handling and to avoid evaporation.

Paragraph beginning at page 66, line 17, was amended as follows:

Sensitivity experiments were carried out as follows: the array was hybridized in [10 L] 10 µL solutions containing progressively decreasing concentrations of labeled target. The

lowest concentration evaluated was 1 fM. At various times, the array was taken out of the hybridization solution, rinsed, and a fluorescence image was collected. The array was then placed back into the hybridization buffer. After hybridization, the array was dehybridized with formamide and five background measurements were taken in 6X SSPE. ROI's from 10 or 100 beads in the five images were averaged to provide the mean background. The mean background values were subtracted from the fluorescence intensities of the various numbers of beads. Individual beads exhibited significant variability such that it was not possible to ascertain whether or not a signal was present. On the other hand, summing signals from multiple beads provided detectable signals. The average signal of ten beads gave a 7% CV while 100 beads provided more precise average values with 3% CV. Results from three representative sets of ten beads for the complementary target and two non-complementary targets are presented in Table 5. The hybridization time was determined when the signal was over three times the standard deviation of the background signals (>3sd). Using this criterion, the microsphere-fiber-optic system is able to detect a 1 fM target solution using a 10 μL volume in 1 hour.

Paragraph beginning at page 66, line 32, was amended as follows:

Both 10 and 100 beads from a total of 500 beads in the array were selected and monitored. In a 1 fM target solution, [10 L] 10 μL contains ca. 6000 DNA molecules. With 500 identical beads in the array giving a signal, each bead would be expected to contain, on average, ca. 12 labeled target molecules on its surface. To confidently attest to the generation of signal, the average signal of at least ten beads was needed. Therefore, this system can give sufficient signal with only 120 molecules.

Paragraph beginning at page 67, line 17, was amended as follows:

Since fluorescein was used to label the DNA targets, we selected encoding dyes with spectral properties that would not overlap with the fluorescein spectrum. Covalently binding

these dyes to the surface of the amine-functionalized microspheres yielded stable and reproducible signals. Unfortunately, such surface encoding reduces the number of amines available for the cyanuric chloride-activated oligonucleotide probe. Therefore, the concentrations of the dyes were optimized to enable sufficient signals from both the encoding dyes and the hybridized target. The finite number of surface amine groups reduces the range and number of dye combinations that can be generated with an external-labeling scheme. To increase the number of encoded microspheres, dyes also can be entrapped inside the bead. Lanthanide dyes are suitable for such internal encoding. The [dyes=] dyes' spectra are not compromised and their intensity remains constant once inside the microsphere.

Paragraph beginning at page 68, line 24, was amended as follows:

The DNA microarray presented here has smaller feature sizes and higher packing densities compared to other DNA arrays. We have demonstrated the fiber optic microarray using a [500 m-diameter] 500 μm-diameter imaging fiber with well diameters of 3.5 m. Fibers have also been tapered to produce nanometer scale wells serving as host to nanometer-diameter beads. Using longer fibers, the microarray sensor tp can be brought to the sample and used to sequentially test multiple solutions. Utilizing the imaging [fiber=s] fiber's remote sensing capabilities, arrays with nanometer dimensions potentially can be used for direct intracellular analysis.

In the Claims:

16. (Twice Amended) A method for increasing the signal-to-noise ratio in the characteristic optical response of [a sensor] an array having subpopulations of sensor elements comprising:

a) providing an array comprising:
i) at least a first subpopulation comprising first sensor elements; and
ii) a second subpopulation comprising second sensor elements;

b) contacting said array with a composition comprising at least a first target analyte;

[a] c) [measuring] obtaining a first measurement from [the optical responses of] at least two of said sensor elements of at least one of said subpopulations [upon exposure to a target analyte];

[b] d) summing [the optical response] said first measurements from said sensor elements; and

[c] e) performing a statistical analysis on said first measurements [of at least one of said subpopulations].

17. (Amended) [A] The method according to claim 16 [wherein prior to said summing, the baseline of at least one optical response signature is adjusted] further comprising obtaining at least a first control measurement and adjusting the baseline of said first measurement against said first control measurement.

18. (Amended) [A] The method according to claim 16 wherein the signal-to-noise ratio is increased by a factor of at least 10.

20. (Amended) The method of claim [15] 16, 25 or 27, wherein said sensor elements are beads and said [sensor] array comprises a population of beads dispersed on a substrate.

25. (Amended) A method for amplifying the characteristic optical response of [a sensor] an array having subpopulations of sensor elements comprising:

a) providing an array comprising:

i) at least a first subpopulation comprising first sensor elements; and

ii) a second subpopulation comprising second sensor elements;

b) contacting said array with a composition comprising at least a first target analyte;

[a] c) [measuring] obtaining a first measurement from [the optical response of] at least two of said sensor elements of at least one of said subpopulations [upon exposure to a target analyte]; and

[b] d) summing the optical responses[; and

c) performing a statistical analysis on said measurements of at least one of said subpopulations].

26. (Amended) A method according to claim 25 [wherein prior to said summing, the baseline of at least one optical response signature is adjusted] further comprising obtaining at least a first control measurement and adjusting the baseline of said first measurement using said first control measurement.

27. (Amended) A method comprising:

a) providing an array with a plurality of subpopulations of sensor elements;

b) contacting said array with a composition comprising at least a first target analyte;

[b] c) [measuring] obtaining first and second measurements from [the optical response of each] at least first and second sensor elements, respectively, from at least a first subpopulation [upon exposure to a target analyte]; and

[c] d) performing a statistical analysis on said first and second measurements [of at least one of said subpopulations].

31. (Amended) The method according to claim [16, 25 or 27] 20, further comprising determining outlying beads and excluding outlying beads from said subpopulation.

32. (Amended) The method according to claim 16, [25] 45 or 27, wherein said statistical analysis comprises calculating the mean of at least [one subpopulation] said first and second measurements.

33. (Amended) The method according to claim 16, [25] 45 or 27, wherein said statistical analysis comprises calculating the standard deviation of at least [one subpopulation] said first and second measurements.

34. (Amended) The method according to claim 16, [25] 45 or 27, further comprising evaluating the statistical validity of said measurements.

35. (Amended) The method according to claim 16, [25] 45 or 27, further comprising performing a second statistical analysis on said measurements.

38. (Amended) The method according to claim 16, [25] 45 or 27, further comprising comparing said statistical analysis of measurements obtained from at least two subpopulations.

40. (Amended) A method comprising:

a) providing an array comprising beads on a substrate comprising a plurality of subpopulations of sensor elements, wherein each sensor element comprises a bioactive agent that will bind a target analyte, and at least two of said subpopulations comprise different bioactive agents that will bind the same target analyte;

b) contacting said array with a composition comprising at least a first target analyte;

[b] c) [measuring] obtaining a measurement from the optical response of each sensor element; and

[c] d) performing a statistical analysis on said measurements from each sensor element [of at least one of said subpopulations].